Neuroprotective Effects of Garlic A Review

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Abstract

Garlic has been investigated extensively for health benefits, resulting in more than one thousand publications over the last decade alone. It is considered one of the best disease preventive foods, based on its potent and varied effects. Midlife risk factors for cardiovascular diseases, such as high serum total cholesterol, raised LDL, increased LDL oxidation, increased platelet aggregation, impaired fibrinolysis, hypertension and homocystinemia are important risk factors for dementia in later years. These risk factors play a major role in the genesis of atherosclerosis of vital arteries causing both cardiovascular and cerebrovascular disease. Garlic is best known for its lipid lowering and anti-atherogenic effects. Possible mechanisms of action include inhibition of the hepatic activities of lipogenic and cholesterogenic enzymes that are thought to be the genesis for dyslipidemias, increased excretion of cholesterol and suppression of LDL-oxidation. Oxidative stress caused by increased accumulation of reactive oxygen species (ROS) in cells has been implicated in the pathophysiology of several neurodegenerative diseases including Alzheimer's disease (AD). Several studies have demonstrated the antioxidant properties of garlic and its different preparations including Aged Garlic Extract (AGE). AGE and S-allyl-cysteines (SAC), a bioactive and bioavailable component in garlic preparations have been shown in a number of in vitro studies to protect neuronal cells against beta-amyloid (A) toxicity and apoptosis. Thus the broad range of anti-atherogenic, antioxidant and anti-apoptotic protection afforded by garlic may be extended to its neuroprotective action, helping to reduce the risk of dementia, including vascular dementia and AD.

Key words: garlic, anti-atherogenic, anti-oxidant, anti-apoptotic, neuroprotective

Introduction

Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases, atherosclerosis, hyperlipidemia, thrombosis, hypertension, dementia, cancer and diabetes [1]. The medicinal use of garlic has a long history. Over the centuries, garlic has acquired a special position in the folklore of many cultures as a formidable prophylactic and therapeutic medicinal agent [2]. Its uses as a remedy for heart disease, tumors, and headaches are documented in the Egyptian Cordex Ebers dating from 1550 BC (2, 3). Garlic is mentioned in the Bible and has been a traditional treatment in many countries notably the Near East, China, and India [2]. Garlic has attracted particular attention of modern medicine because of its widespread use around the world and the cherished belief that it helps to maintain good health by warding off illness and providing more vigor.

Recently, health benefits of garlic as a neuroprotective agent are beginning to emerge [4]. This review discusses the possible mechanisms of therapeutic actions of garlic and its preparations. The neuroprotective effects of garlic may be attributed to its three interrelated anti-atherogenic, antioxidant and anti-apoptotic properties.

Garlic preparations and their bioactive constituents

Raw garlic homogenate has been the major preparation of garlic subjected to intensive scientific study, because it is the most common method of garlic consumption. Raw garlic homogenate is essentially the same as the aqueous garlic extract which has been used in various scientific studies. Allicin (allyl 2-propene thiosulfinate or Diallyl thiosulfinate was long thought to be the principal bioactive compound present in aqueous extract or raw garlic homogenate [5]. When garlic is chopped or crushed, allinase enzyme, present in garlic is activated and acts on alliin (present in whole garlic) to produce allicin [6]. Other important sulfur-containing compounds present in garlic homogenate are allyl methyl thiosulfonate, 1-propenyl allyl thiosulfonate and -L-glutamyl-S-alkyl-L-cysteine [7]. The enzyme allinase responsible for converting alliin (S-allyl cysteine sulfoxide) to allicin is inactivated by heat [1]. Thus the water extract of heat treated garlic contains primarily alliin. Although thiosulfinates such as allicin have long been thought to be active compounds due to the characteristic odor, it is not necessary for garlic preparations to contain odorous compounds to be effective. They decompose and disappear during any processing [8].

Garlic products have become more popular in the last decade. Market research conducted in United States (1998) showed that garlic products were the most popular of all 91 dietary supplements [9]. Dozens of brands on store shelves can be classified into four groups: garlic oil, garlic oil macerate, garlic powder and aged garlic extract (AGE).

Garlic oil: Medicinally used garlic oil is prepared by steam distillation process. Distilled garlic oil consists of a variety of sulfides such as diallyl disulfide (DADS) and diallyl trisulfide (DAT) [6]. Whole garlic cloves ground in water are distilled by heat or extracted by an organic solvent (i.e. hexane) to obtain fractionated oil. Water soluble compounds are totally eliminated by this process. Allicin is also completely eliminated from the oil [1].

Garlic oil macerate: Oil macerate products are made of encapsulated mixtures of whole garlic cloves ground into vegetable oil. During the manufacturing process, some alliin is converted to allicin. Because allicin is unstable and decomposes quickly, oil macerate preparations contain allicin – decomposed compounds such as dithiins, ajoene

and sulfides, residual amounts of alliin and other constituents in garlic [10].

Garlic powder: Garlic cloves are sliced or crushed, dried and pulverized into powder. The main sulfur compound in both raw garlic and garlic powder is alliin [11]. Garlic powder contains no allicin, possibly accounting for its instability [12].

Aged garlic extract: Another widely studied garlic preparation is AGE. Sliced raw garlic stored in 15-20% ethanol for 20 months is referred to as AGE. This whole process is supposed to cause considerable loss of allicin and increased activity of certain newer compounds such as S-allylcysteine (SAC), S-allyl mercaptocysteine, allixin, saponins and selenium which are stable, highly bioavailable and significantly antioxidant. SAC is one of the most active ingredients in AGE [13]. SAC is a safe compound and its biological effects are well researched. The US National Cancer Institute tested SAC toxicity as compared to other typical garlic compounds and found that SAC is less toxic than allicin and DADS [13].

The neuroprotective effects of garlic

The nervous system is the major communication network in the human body. Its normal functioning is strongly dependant on the maintenance of its structural integrity and many complex metabolic processes.

Accordingly, the processes that disrupt normal structure or metabolism, or both, are capable of producing neurological disease. The degenerative diseases of the central nervous system (CNS) encompass a heterogeneous group of disorders characterized by spontaneous, progressive degeneration of neurons in specific regions of the brain, spinal cord, or both. The neurodegenerative disorders include Alzheimer's Disease, Parkinson's Disease, Huntington's Disease and Amyotrophic Lateral Sclerosis (motor neuron disease). AD is the most common cause of dementia in the elderly with cerebrovascular disease and several less common neurodegenerative disorders accounting for most of the remaining cases [14].

Antiatherogenic- neuroprotective effects of garlic

Atherosclerosis is a complex disease, characterized by an extensive inflammatory, fibro-fatty, proliferative response to damage of the arterial wall involving several cell types, particularly muscle cells, monocyte-derived macrophages, T-lymphocytes and platelets [15]. Hyperlipidemia constitutes a major pathological factor that predisposes for atherosclerosis. The medicinal value of garlic is best known for its lipid lowering and anti-atherogenic effect [16].

Cardiovascular disease is associated with multiple risk factors such as raised serum total cholesterol, elevated low density lipoprotein (LDL) and an increase in LDL oxidation, increased platelet aggregation, impaired fibrinolysis, hyper homocystinemia, and hypertension [17].

Growing evidence supports a strong and likely causal association between cardiovascular disease (CVD) and its risk factors with incidence of cognitive decline and AD [18-21]. Individuals with subclinical CVD are at higher risk for dementia and AD [18]. Based on both cross sectional and longitudinal epidemiology studies, there are statistically significant correlations between the prevalence of AD and several other diseases or symptoms. These include diabetes, hypercholesterolemia, hypertension, hyperhomocysteinemia, dietary intake of saturated fats, elevated cholesterol, alcohol consumption, smoking, physical inactivity, atrial fibrillation, atherosclerotic disease, and plasma concentrations of some hemostatic factors [19]. Newman et al [20] have observed that the incidence of dementia was higher in those with CVD, particularly in the subgroup with peripheral artery disease (PAD) suggesting that extensive peripheral atherosclerosis is a risk factor for AD. Bergman C et al [21] recently reviewed both observational and clinical studies regarding the association between anti-hypertensive, lipid lowering and anti-diabetic medications and the risk of impaired cognition, dementia or AD. They proposed that early interventions at reducing these cardiovascular risk factors may have an impact on future incidence of several cognitive deficits including AD.

Next to AD, vascular dementia is the second most common form of senility in the elderly suggesting that dyslipidemia may be relevant to the occurrence of dementia with a vascular component [22]. Studies have also found that baseline high density lipoprotein levels were lower and triglyceride levels were higher in elderly men who developed dementia with vascular components [18].

The risk of cognitive decline in community dwelling was highest among APOE4 allele carriers who had high cholesterol levels, high fibrinogen levels, or diabetes [23]. Saczynski et al [24] recently raised the possibility that various lipoprotein components of cholesterol may be differentially associated with dementia. In addition to the role of cholesterol in Aβ generation, interactions of cholesterol with Aβ and its role in the pathogenesis of AD have been shown i.e. Aβ affects cholesterol dynamics in neurons, and altered cholesterol metabolism in turn leads to neurodegeneration with abnormally phosphorylated tau [25].

The pathways from elevated LDL cholesterol to the development of dementia or stroke are unclear, but promotion of atherosclerosis by LDL-cholesterol may provide a link. High concentrations of LDL-cholesterol are known to be independently associated with coronary heart disease [17] and carotid artery atherosclerosis [26] which in turn may lead to cognitive decline through cerebral embolism or hypoperfusion [27]. A recent epidemiological study by Kin et al [28] provides compelling evidence that carotid atherosclerosis is associated with brain atrophy. The authors suggest that carotid atherosclerosis may be a useful morphological index of brain atrophy.

Lipid peroxidation may also be a major factor in the aging process and hypercholesterolemic diets may lead to microglial activation [29] and beta-amyloid [A] plaque deposition (30). Thus cholesterol oxidation in the brain may be particularly relevant to the pathogenesis of vascular dementia with mixed pathology (i.e. AD with concomitant stroke).

In our laboratory we demonstrated that the metabolism of glycosaminoglycans (GAGs) is affected in

hypercholesterolemia with increased sulfated fractions [31] Inclusion of garlic protein in the diet of cholesterol fed rats significantly decreased the concentration of total GAG in the heart and aorta, especially of the sulfated fractions. This is possibly due to the increased activity of GAG degrading enzymes such as β-glucuronidase, β-N-acetyl hexosaminidase aryl sulfatase and hyaluronidase. Concentration of total GAG was measured by estimating uronic acid concentration in the papain digestion of dry defatted tissue. GAG fractions were estimated in the total GAG solution after digestion with hyaluronidase and chondroitinase ABC followed by ion exchange separation and estimation of uronic acid in the elutes. The activity of β-glucuronidase and β-N acetyl hexosaminidase was assayed by measuring the release of paranitrophenol from paranitrophenol β –D-glucuronide and paranitrophenol –β-acetylglucoseamide. Hyaluronidase activity was assayed by estimating N-acetylhexoseamine liberated from hyaluronic acid. Aryl sulfatase was assayed by measuring the liberated catechol from paranitrocatechol sulfate.

Sulfated GAGs are implicated in lipid accumulation in developing lesions because of their ability to bind plasma lipoproteins, especially LDL. The neurotoxic effects of several amyloidogenic peptides including A in AD is potentiated by increased sulfated GAGs [32].

Hypertension may contribute to cognitive decline seen in AD by causing cerebral small vessel pathology and increasing the number of neurofibrillar tangles and amyloid plaques [33].

Elevated plasma homocysteine is an independent risk factor for cardiovascular disease, stroke and dementia including AD [34]. Homocysteine thiolactone, a highly reactive free radical which thiolates LDL particles is formed in hyperhomocysteinemia. The modified LDL particles tend to aggregate, are endocytosed by macrophages, and increase the tendency for atherogenesis [34].

Platelet aggregation superimposed on an atherosclerotic vessel is an antecedent event causing total blockage of blood flow leading to thromboembolic diseases, hypoperfusion, stroke, and dementia [15].

The medicinal value of garlic is best known for its lipid lowering and antiatherogenic effects. In animal experiments, garlic extracts have been shown to lower plasma lipid and cholesterol in rats, rabbits, chickens and swine [35-41].

Moreover a number of studies have similarly shown that garlic and garlic preparations significantly reduced plasma lipids, especially total cholesterol and LDL-cholesterol in humans [42-45]. Aside from the reported antiplatelet aggregation, garlic stimulated fibrinolytic activity [46-52].

The protective effect of garlic on atherosclerosis has been attributed to its capacity to reduce lipid content in arterial wall. Animal studies have shown that supplementation of garlic in the diet depressed the hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, glucose-6-phosphate dehydrogenase and 3 hydroxy-3-methylglutarylCoA (HMG CoA) reductase [36].

The activity of hepatic G6PD and malic enzyme was assayed by measuring the rate of formation of NADPH using glucose -6- phosphate and malate respectively as substrates. The activity of hepatic HMG –CoA reductase was determined by the ratio of HMG-CoA to mevalonic acid in fresh rat liver homogenate. The lower the ratio is the higher the enzyme activity. Studies in our laboratory and elsewhere have shown the formation of protein internal disulfides by thiol disulfide exchange reaction to be the cause of this inactivation [53, 54]. This was demonstrated by identifying the interaction between sulfur compounds and garlic components using chromatographic methods and by tryptic peptide analysis.

Gebhhardt et al [55] and Yeh et al [56] have reported the multiple inhibitory effects of garlic extracts on cholesterol biosynthesis in cultured rat hepatocytes. The inhibition of HMG-CoA reductase by garlic has also been confirmed in a recent study from our laboratory [38]. In animal studies, we have also demonstrated that administration of garlic increased the hepatic degradation of cholesterol to bile acids [37]. Bile acids were estimated by the enzymatic procedure using 3α hydroxy steroid dehydrogenase and monitoring the rate of formation of NADH using cholic acid as standard.

Mehrzia et al [57] examined the acute effects of a partially purified fraction from garlic on plasma glucose and cholesterol levels in rats, and confirmed that aqueous extract of garlic contained an active fraction different from S-allyl cysteine sulfoxide, exerting both glucose and cholesterol lowering activity. Control (vehicle H2O) or garlic extract treated groups at 100 – 120 mg protein / kg body weight were intraperitoneally injected, and glucose, cholesterol, insulin, and nitric oxide metabolite levels were determined after short term duration of six hours. The mechanism of action seemed to involve nitric oxide (NO), which increased time and dose dependently. The garlic effects were abolished by diphenyleneiodonium chloride (DPI = 1 mg / kg body weight), a specific inhibitor of nitric oxide production, suggesting the involvement of constitutive nitric oxide synthesis. LDL isolated from human subjects given AGE and aqueous garlic extract was found to be significantly more resistant to oxidation (58). Allicin was identified initially as the compound responsible for anti-atherosclerotic effect [5]. However recent in vitro studies revealed that water soluble organic sulfur compounds, especially SAC present in AGE and DADS present in garlic oil, are also potent inhibitors of cholesterol synthesis [8]. AGE has consistent effects on risk factors for CVD including cholesterol [59, 60].

In some of these studies, blood SAC level was measured in the subjects as a compliance marker. The blood SAC level in the group taking supplements was significantly higher than that of the placebo group [60, 61]. It is clear that SAC is bioavailable because it was absorbed into the blood and is therefore active in the human body. Budoff et al have demonstrated that AGE retards the progress of coronary artery calcification and Weiss et al reported that AGE improves homocysteine-induced endothelial dysfunction in macro- and micro-circulation [62, 63]. Yeh et al [64] have reported that garlic extract attenuates hyperhomocysteinemia. Increased amounts of homocysteine exerts several pro-atherosclerotic effects,

including damaging the endothelium of blood vessels. Thus the decrease in levels of elevated homocysteine by garlic ingestion suggests its protective role against CVD.

The formation of atherosclerotic plaque leads to narrowing of vessel walls when proliferative changes occur. This proliferation is due to liberation of various growth factors by macrophages and platelets [65]. Platelet aggregation superimposed on atherosclerotic vessel leads to thrombosis (coronary, cerebral or peripheral vascular) leading to ischemia of the tissue. Srivastava et al [49] reported that aqueous extract of garlic inhibits platelet aggregation induced by ADP, epinephrine, collagen, and calcium ionophore A23187 in a dose dependant manner. Sendl et al [66] demonstrated that chloroform / acetone extracts of fresh garlic inhibit cyclo-oxygenase activity directly in cell-free assays, with the acetone extract being more effective. Ajoene, another garlic derivative, has been shown to inhibit in vitro platelet aggregation in different animals, i.e. cow, dog, guinea pig, horse, monkey, rabbit, and rat [46].

Teraniski et al [47] have also demonstrated that ajoene inhibits platelet aggregation in vitro. Chan et al [67] in a recent study have shown that diallyl trisulfide (DAT) rich garlic oil (GO) supplement at 5 or 50 mg/kg body weight when fed to Sprague Dawley rats significantly prolonged bleeding time and thrombin time and enhanced anticoagulation factor activity, such as antithrombin III and protein C. The authors postulated that the anticoagulant action of DAT-rich GO was due to inhibition and/or inactivation of thrombin. Another garlic component, sodium 2-propenyl thiosulfate was found to modulate cyclo-oxygenase activity in canine platelets thus preventing their aggregation [48]. The studies of Qi et al [68] demonstrated that the mechanism of inhibition of platelet aggregation by garlic constituents may also be via the inhibition of calcium mobilization. The antiplatelet aggregation mechanism of garlic has been attributed to reduced formation of thromboxanes, inhibition of phospholipase activity, and lipoxygenase products found in platelets [49]. Garlic has a positive response in the inhibition of platelet aggregation in both healthy subjects and subjects with cardiovascular disease [61,69,70]. Fibrinolysis is also enhanced by garlic, resulting in dissociation of clots and thrombi.

In human studies, Bordia et al [50-52] consistently showed the positive fibrinolytic activity of garlic. They reported that chronic (three weeks to three months) administration of GO increased fibrinolytic activity ranging from 36% to 130% in healthy patients as well as patients who had had an acute myocardial infarction [50, 51]. Studies by other investigators have also found the same results [71, 72]. Epidemiological studies support a strong association between hypertension, CVD, and dementia [18, 19]. The antihypertensive effects of garlic have been studied but remain controversial. In a 1994 meta-analysis by Silagy et al [73] assessing the effect of garlic on hypertension, three trials showed significant reductions in systolic blood pressure (>7.7 mm Hg reduction), and four trials showed reduction in diastolic blood pressure (>5 mm Hg reduction) with garlic treatment compared with placebo. All the trials used the same dried garlic powder preparation (Kwai). This meta-analysis suggests that the garlic powder preparation may be of some clinical use in subjects with mild hypertension. In a more recent meta-analysis [74] 23 placebo-controlled trials were analyzed. Only three trials showed a statistically significant reduction in diastolic blood pressure, and one showed a statistically significant reduction in systolic blood pressure in patients treated with garlic compared with placebo. Rashid et al [75] suggested that the mechanism of antihypertensive action of garlic is due to its prostaglandin-like effects, which decrease peripheral vascular resistance. The gamma-glutamyl cysteines are the compounds in garlic that may lower blood pressure, as indicated by their ability to inhibit angiotensin-converting enzyme in vitro [66].

Aortic stiffening is as much an important risk factor in cardiovascular morbidity and mortality and cerebrovascular incidents. Elevated aortic stiffness induces high systolic blood pressure, augmented pulse pressure with increased ventricular afterload, reduced subendocardial blood flow and augmented pulsatile stress in the peripheral arteries [76]. In populations consuming garlic for long periods (≥ 300 mg of garlic powder/day for ≥ 2 years) attenuation of an age-related increase in aortic stiffness has been observed [77]. This suggests a protective effect on the elastic properties of the aorta related to aging in humans. Ahmad et al [78] have recently reported that AGE and SAC prevent formation of advanced glycation end products (AGEs). Accumulation of AGEs lead to crosslinking of matrix proteins with altered function. Amagase et al [8] reported that among the different garlic products, AGE which is odorless and rich in antioxidants was found to be very effective to modulate cardiovascular risk factors in both clinical and preclinical settings.

Table 1 The key points about the proposed mechanism of antiatherogenic and related effects of garlic.

• Depressed hepatic activities of lipogenic and cholesterogenic enzymes
• Increased hepatic degradation of cholesterol to bile acids
• Decreased plasma LDL- cholesterol levels
• Antithrombotic and anti-platelet aggregation effects due to reduced formation of thromboxanes, inhibition of phospholipase activity, lipoxygenase products formation in platelets and of calcium mobilization
• Stimulates fibrolytic activity
• Lowers plasma homocysteine levels
• Antihypertensive action and decrease in peripheral vascular resistance due to its prostaglandin like effects and ability to inhibit angiotensin–converting enzyme
• Attenuation of aortic stiffness and thus protective effect on the elastic properties of the aorta

Human clinical trials on antiatherogenic and related effects of garlic

The Agency for Healthcare Research and Quality [AHRQ] in their evidence report dated October, 2000, [79] summarized the effects of garlic on cardiovascular risk factors and disease, the association between garlic and cancer, and possible adverse effects of garlic.

English and non-English citations were identified through February, 2000, from 11 electronic databases, references of pertinent article reviews, manufacturers, and technical experts. They limited the review of cardiovascular- related effects to randomized, controlled trials in humans that lasted at least four weeks and compared garlic with placebo, no garlic, or another active agent. There were 45 randomized controlled trials (RCTs) lasting at least four weeks that tested the effects of garlic on cardiovascular-related end points. The results indicate that 37 randomized trials (all but one in adults) consistently showed that compared with placebo, various garlic preparations led to small statistically significant reductions in total cholesterol at one month (range of average pooled reductions 1.2 to 17.3 milligrams per deciliter [mg/dL]) and three months (range of average pooled reductions 12.4 to 25.4 mg/dL). Eight trials with outcomes at six months showed no significant reductions of garlic compared with placebo. Changes in LDL levels and triglycerides mirrored total cholesterol results; no significant changes in HDL levels were found. Twenty-seven small, randomized, placebo-controlled trials (all but one in adults) reported mixed but never large effects of various garlic preparations on blood pressure outcomes. Ten small trials (all but one in adults) of short duration showed the effects of various garlic preparations on platelet aggregation, and mixed effects on plasma viscosity and fibrinolytic activity.

Rahman et al [16] critically reviewed human trials which were conducted since 1993. Only those trails which were conducted for a minimum period of two weeks and that addressed the following parameters had been included: (a) cholesterol- lowering effects, (b) inhibition of platelet aggregation (c) lowering of blood pressure and (d) other cardioprotective properties. They reported that since 1993, 44% of the clinical trials have indicated a reduction in total cholesterol and all the seven clinical trials on the inhibition of platelet aggregation showed positive response in both healthy subjects and subjects with CVD . Mixed results were obtained in the area of blood pressure and oxidative stress reduction where very few trials addressed these issues. The different composition and quality of sulfur compounds of different garlic preparations, unknown active constituents and their bioavailability, inadequate randomization, selection of inappropriate subjects, and the short duration of trials could account for the inconsistent findings.

(1998–2007) showing the hypolipidemic effects of garlic. Thus with the emerging literature on the association between traditional cardiovascular risk factors, dementia, and AD, the anti-atherogenic and related protection afforded by garlic may be extended to its neuroprotective action, helping reduce the risk for cerebrovascular disease and dementia. However, further well-designed randomized, larger clinical trials of longer duration using well standardized preparations of garlic need to be conducted to support these effects.

Table 2 gives a summary of major clinical trials during the last decade (1998-2007)

Antioxidant-neuroprotective effects of garlic

Reactive oxygen species (ROS), or free radicals, have been implicated in mediating various pathological processes such as cancer, cardiovascular, neurodegenerative and inflammatory diseases [80]. Oxidative stress caused by increased accumulation of ROS in cells has been implicated in the pathophysiology of several neurodegenerative diseases including AD [81]. Common pathogenetic mechanisms for both atherosclerotic disease and AD, such as inflammation and the generation of ROS suggest a causal link [19]. Thus identification of modifiable risk factors for dementia becomes a research priority and early intervention aimed at reducing those cardiovascular risk factors a therapeutic imperative.

ROS include free radicals and non-radical species. The free radicals carry an unpaired electron and are unstable and reactive. They include superoxide, nitric oxide and the most reactive and toxic ROS, the hydroxyl radical. Non-radical oxidants include hydrogen peroxide, singlet oxygen, and ozone, which form free radicals through various chemical reactions. The reactions relevant to cell injury mediated by free radicals include lipid peroxidation of membranes, DNA fragmentation, and cross-linking of proteins.

However, cells have also developed several enzymatic and non-enzymatic systems to inactivate free radicals. The major antioxidant enzymes are superoxide dismutase (SOD), catalase, and glutathione peroxidase [8]. Free radicals are neutralized by scavengers (vitamins E, A, C and carotene), and the ability of Cu++ and Fe+++ to form free radicals is minimized by binding the ions to carrier proteins (ferritin and ceruloplasmin respectively).

Garlic has been reported to be effective against diseases of which ROS are considered to be the main cause [8,58]. Oxidative modification of LDL increases the risk of atherosclerosis, cardiovascular and cerebrovascular disease. Oxidized LDL acquires new antigenic properties that are recognized by the host immune system as "foreign". Thus oxidized LDL produces several new biologic responses which contribute to the thickening and narrowing of arteries, the principal event in atherosclerosis [82]. Borek [80] and Amagase [8] have cited several studies which have demonstrated the antioxidant properties of garlic. Raw garlic homogenate augmented endogenous antioxidants along with reduction of basal lipid peroxidation in rat heart, liver and kidney in a dose dependant manner [83]. Lau et al [82] provided experimental evidence showing that several garlic compounds can suppress LDL oxidation in vitro. Short term supplementation of garlic in human subjects has demonstrated an increase resistance of LDL to oxidation. Their data suggest that suppressed LDL oxidation may be one of the mechanisms that accounts for the beneficial effects of garlic in cardiovascular health. AGE exerted its antioxidant action by scavenging reactive oxygen species and enhancing the cellular antioxidants like reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) of vascular endothelial cells [84,85]. Popov et al [86] observed the antioxidant effect of the aqueous extract from a

Table 2 A summary of major clinical trials during the last decade (1998-2007)

	Durak et al (2004)	Turner et al (2004)	Kannar et al (2001)	Yeh et al (2001)
Method	Not placebo controlled	Double-blind placebo controlled	Double-blind placebo controlled	Double-blind placebo controlled
Preparation	AGE	Garlic Powder	Garlic Powder	AGE
Subject Selection	Hypercholesterolemic	Healthy	Hypercholesterolemic	Hypercholesterol-emic
No. of subjects	n= 23	n= 75	n= 46	n= 34
Duration	4 mo	12 wks	12 wks	5 mo
Results	↓ TC ↓ LDL-C ↓ VLDL-C ↓ TAG	↓ TAG no change in TC, LDL-C, HDL-C	↓ TC ↓ LDL-C no change in TAG, HDL-C	↓ TC ↓ LDL-C no change in TAG, HDL-C

dehydrated garlic powder preparation by using photochemiluminescence on the Cu2+ initiated oxidation of LDL. The formation of conjugated diene, which accompanies the lipid peroxidation process, was detected photometrically. AGE and its constituent SAC have a similar preventive effect against Cu2+ initiated oxidation of LDL taken from human subjects who consume AGE [87]. Horie et al [88] have demonstrated that AGE prevents the formation of thiobarbituric acid reactive substances (TBARS) and fluorescent substances during lipid peroxidation of rat liver microsomes. Imai et al (84) compared the antioxidant properties of three garlic preparations and organosulfur compounds in garlic. AGE inhibited the emission of low level chemiluminescence and the early formation of TBARS in a liver microsomal fraction initiated by t –butyl hydroperoxide. However, the water extracts of raw and heat treated garlic enhanced the emission of low level chemiluminiscence. In vitro studies have shown that AGE improves circulation and blood properties by preventing lipid peroxidation and hemolysis in oxidized erythrocytes [89].

Nuclear factor –κ (NF-κB) is a transcription factor that is regulated by the redox state of the cell and implicated in the inducible expression of a variety of genes involved in oxidative stress and cellular responses to stress. NF-κB is thought to play a role in atherogenesis because minimally modified LDL has been shown to activate NF-κB activation [90]. Geng et al [91] have demonstrated that AGE and SAC inhibit TNF-α and hydrogen peroxide induced activation of NF-κB in human T cells. Inhibition of NF-κB by AGE in part by preventing oxidative modification of LDL, further supports the role of AGE in helping to prevent atherogenesis and lowering the risk of heart disease and stroke. Gorinstein et al [92] investigated and found that both raw and boiled garlic enhances plasma antioxidant activity and improves plasma lipid metabolism in cholesterol fed rats. It was found that garlic boiled for 20 minutes has the same bioactivity as raw garlic in its antioxidant and protein spectra. The selenium and copper content of raw garlic is not altered by boiling.

Pari et al [93] have recently demonstrated that the garlic compound diallyltetrasulfide [DTS] has cytoprotective and antioxidant activity against cadmium [Cd] induced toxicity. Cd is a neurotoxic metal, which induces oxidative stress and membrane disturbances in nerve system. In rats exposed to Cd (3 mg/kg/day) subcutaneously for 3 weeks, a significant increase in the levels of lipid peroxidation (LPO) and protein carbonyls along with a significant decrease in the levels of GSH and total sulphydryl groups (TSH) and the activities of acetylcholinesterase (AChE), SOD, CAT, G Px, GST, membrane bound enzymes (ATPases: Na+ K+-ATPase , Mg2+-ATPase and Ca2+ATPase) were observed in the brain tissue. Oral administration of DTS (40 mg/kg/day) with Cd significantly diminished the levels of LPO and protein carbonyls and increased the activity of ATPases and antioxidant enzymes in the brain. These results suggest that DTS protects the brain function from the toxic effects of Cd.

In another study, Pari et al [94] have reported the cytoprotective and antioxidant role of DTS on Cd- induced renal injury. In vitro studies with kidney cell lines (vero cells) showed that incubation of DTS (5–50 µg/ml) with Cd (10 µM) significantly reduced the cell death induced by Cd (µM) indicating its cytoprotective property. Further, the flow cytometric assessment on the level of intracellular reactive oxygen species using a fluorescent probe 2' 7'dichlorofluorescein diacetate (DCFDA) confirmed the Cd induced intracellular oxidative stress in vero cells, which was significantly suppressed by DTS (40 µg/ml). These antioxidant cytoprotective effects of compounds from garlic against Cd toxicity attain great significance from the fact that several reports suggest the role of heavy metals in neuronal degeneration [95,96].

Liu et al [97] reported that GO prevents tributyltin (TBT) induced oxidative damage in vivo and in vitro. They found that both reactive oxygen species (ROS) production and malondialdehyde (MDA) content decreased in mice pretreated with GO. The number of cells with damaged DNA in unprotected mice increased significantly compared with that in GO protected mice. In human FL (human amniotic cells) cell studies, TBT induced intracellular ROS generation was significantly inhibited after FL cells were

pretreated with GO, and the TBT induced cytotoxic effects were also prevented by GO.

The high antioxidant level in AGE helps prevent the oxidant damage that occurs during ischemia or reperfusion. Ischemia followed by reperfusion results in an increased production of free radicals and oxidant stress that may lead to neuronal death by apoptosis that contributes to the development of dementia following stroke.

Saleem et al [98] have reported that AGE effectively modulates neuro-behavioural and neuro-chemical changes in focal ischemia, most probably by virtue of its antioxidant properties. In their study, the middle cerebral artery [MCA] of male Wistar rats was occluded for 2 hours using intraluminal 4-O monofilament and reperfusion was allowed for 22 hours. Middle cerebral artery occlusion (MCAO) caused significant depletion in GSH and its dependant enzymes (GPx, GR and GST) and significant elevation of MDA, glutamate, and aspartate. The activities of Na +, K+, ATPase, SOD, and CAT were decreased significantly by MCAO. The neuro-behavioural activities (group strength, spontaneous motor activity, and motor coordination) were also decreased significantly in the MCAO group. All of the alterations induced by ischemia were significantly attenuated by pretreatment with AGE (500 mg/ml/kg body wt., i.p) 30 minutes before the induction of MCAO and correlated well with histopathology by decreasing the neuronal cell death following MCAO and reperfusion. In a similar study, Gupta et al [99] observed the neuroprotective effects of GO on ischemia and reperfusion induced cerebral injury. Global cerebral ischemia was induced by occluding right and left common carotid arteries for ten minutes followed by reperfusion for 24 hours. Cerebral infarct size was estimated using triphenyltetrazolium staining. Mitochondrial TBARS assay was employed as an index of oxidative stress. Administration of GO before global cerebral ischemia markedly reduced cerebral infarct size and attenuated impairment in short term memory and motor coordination. The protective effects of AGE was reported in a preclinical study of ischemia, and the findings showed that treatment with SAC attenuated damaging reactive oxygen species and prevented brain injury, reducing infarct volume (100).

These data indicate the antioxidant properties of garlic in preventing myocardial and cerebrovascular incidents and dementia.

Human clinical trials on antioxidant effects of garlic

Antioxidant effect of garlic in humans is not well studied. Rahman et al [16] have recently reviewed the literature on clinical trials related to the antioxidant properties of garlic. Seven studies since 1993 were identified. They reported that five of the studies showed a decrease in oxidative stress [45,101-104] while two studies showed no significant change in oxidative stress parameters [70,105]. Further randomized, placebo–controlled, larger clinical trials need to be conducted to firmly establish the antioxidant properties of garlic.

Table 3 The key points about the mechanism of antioxidant effects of garlic

- Scavenging ROS, inhibiting LDL oxidation
- Protection of endothelial cell integrity by inhibition of lipid peroxidation induced injury
- Inhibits homocysteine thiolactone formation
- Enhancement of cellular reduced glutathione levels (GSH)
- Enhancement of cellular scavenging enzymes such as superoxide dismutase (SOD) catalase (CAT) and glutathione peroxidase (GPx)
- Inhibition of nuclear factor- κB activation
- Modulating neurobehavioral changes in reversible focal ischemia and reperfusion induced cerebral injury by virtue of its antioxidant properties

Table 4 A summary of major clinical trials during the last decade [1998-2007] showing the antioxidant properties of garlic.

	Durak et al (2004)	Durak et al (2004)	Dhawan et al (2004)	Dillon et al (2002)
Method	Not placebo controlled	Not placebo controlled	Not placebo controlled	Not placebo controlled
Preparation	AGE	AGE	Garlic Pearls	AGE
Subject selection	Atherosclerotic	Hypercholesterol-emic	Hypertensive	smoking & non smoking
No. of subjects	n= 11	n= 23	n= 20	n= not specified
Duration	6 mo	4 mo	2 mo	14 days
Results	↓ biomarkers of oxidative stress in blood	↓ biomarkers of oxidative stress in blood	↓ biomarkers of oxidative stress in blood	↓ biomarkers of oxidative stress in blood

AGE Aged garlic extract

Anti-apoptotic-neuroprotective effects of garlic

Apoptosis occurs through two well recognized pathways in cells. Both effector mechanisms of apoptosis are associated with caspase activation and include the intrinsic or mitochondria mediated effector mechanism and the extrinsic or death receptor mediated effector mechanism [106]. The mitochondrial membrane constitutes the battleground on which opposing signals combat to seal the cells fate.

Increased mitochondrial permeability and dissipation of the electrochemical gradient or membrane potential via opening of the mitochondrial permeability transition pore triggers cell death by releasing apoptogenic factors from

within the mitochondria, with subsequent cytochrome C release, caspase activation, and ultimately execution of apoptosis [107]. The release of cytochrome C is believed to be a key event in apoptosis, and it is regulated by the genes of the BCL2 family. Some members of this family (eg. BCL2, BCLXl, BCL-W) inhibit apoptosis by preventing the release of cytochrome C, whereas others such as BAD, BAX, BID and BIM promote apoptosis by favoring cytochrome C release [106]. The mechanism of β amyloid-induced neuronal apoptosis sequentially involves C-Jun N terminal kinase activation, BCL-W down regulation and release of apoptogenic factor second mitochondrial derived activator of caspase, followed by cell death [108]. Recently, Biswas et al [109] have demonstrated that BIM is elevated in AD neurons and is required for -amyloid induced apoptosis.

After exposing rat pheochromocytoma (PC12) cells to Aβ, a significant increase in ROS preceded apoptotic events [110]. AGE, SAC, and DADS have been shown in a number of in vitro studies to protect neuronal cells against A β toxicity and apoptosis [110–112]. AGE and SAC not only suppressed the generation of ROS but also attenuated caspase-3 activation, DNA fragmentation, and eventually protected neurons against Aβ induced apoptosis [110].

Koh et al [112] have studied the effects of diallyl disulfide (DADS), a garlic derived compound, on the viability of neuronal cells and cell signals including phosphatidylinositol 3- kinase (P13 K), glycogen synthase kinase-3 (GSK-3) cytochrome c, caspase -3 and poly (ADP-ribose) polymerase (PARP) in PC12 cells neuronally differentiated by nerve growth factor. To evaluate the protective effects of DADS on oxidative stress–induced nPC12 cells, the viability of the cells (pretreated with DADS for two hours vs not pretreated) was evaluated 24 hours after exposure to100 μM H2O2 for 30 min. Compared to the cells treated with 100μM H2O2 only, pretreatment of the cells with 20μM DADS before exposure to 100μM H2O2 increased the viability and induced activation of P13K, inactivation of GSK-3 and inhibition of cytochrome C release, caspase-3-activation and PARP cleavage. These results indicate the neuroprotective effect of DADS by its anti-apoptotic properties.

In a recent study Chauhan et al [113] have reported the amelioration of early cognitive deficits by AGE in Alzheimer's transgenic mice. Feeding of AGE prevented deterioration of hippocampal-based memory tasks in these mice, suggesting that AGE has a potential for preventing AD progression. Thus the anti-apoptotic properties of garlic and its constituents may also contribute to its neuroprotective effect.

Contraindications, adverse effects, interactions

Animal studies with rats in our laboratory have shown that prolonged feeding of high levels of raw garlic in rats has resulted in anemia, weight loss, and failure to grow due to lysis of red blood cells [114]. Raw garlic juice at a dose of 5 ml/kg has resulted in death due to stomach injury [115]. Chronic administration of garlic powder (50 mg/day) resulted in inhibition of spermatogenesis in rats. Reduced concentration of sialic acid in the testes, epididymis and seminal vesicles together with decreased leydig cell function reflects anti-androgenic effects of garlic [116]. All the above mentioned toxicity reports cannot be explained to the full extent but for interactions of specific enzymes with the sulfoxides. Relatively few side effects were reported in human clinical trials using garlic and its preparations. Most of the reported side effects were nonspecific. The ingestion of one or two cloves of garlic per day is considered safe in adults [117]. The most common side effect of ingested garlic is offensive breath and body odor. Consumption of excessive amounts of garlic, especially on an empty stomach, can cause gastrointestinal upset, flatulence, and changes in the intestinal flora [74, 118]. There have been reports of allergic dermatitis, burns, and blisters from topical application of raw garlic. Whether adverse effects occur more commonly with certain preparations than others was not established. Furthermore, the causality of the adverse effects was unclear, except for breath and body odor, and the expected frequency of adverse effects was not determined. Garlic appears to have no effect on drug metabolism [119], although recent studies in healthy volunteers show conflicting results related to garlic's effect on protease inhibitor pharmacokinetics [120,121]. A few reports of adverse effects related to bleeding and interaction with other drugs such as warfarin and aspirin have also been reported [122,123]. It has been suggested that patients taking anticoagulants use caution when taking garlic because of its anti-thrombotic properties [74,120]. It seems prudent to stop taking high doses of garlic seven to ten days before surgery because garlic can prolong bleeding time and has been associated (in one case report) with spontaneous spinal epidural hematoma [74,124].

Conclusion

Garlic has many health benefits and has been traditionally used worldwide over the centuries. The wealth of scientific literature supports the proposal that garlic and its preparations help in preventing or reducing the risk of cardiovascular ailments, stroke, and cancer. Recently the beneficial effects of garlic and its constituents on neuronal physiology and brain function are beginning to emerge. This review encompasses multiple health effects of garlic and its constituents with references to neuroprotection. Further studies should be carried out to identify specific compounds from garlic that are responsible for most of its biological effects.

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